EXPERIMENTAL CYTOLOGICAL EVIDENCE FOR AN OUTWARD SECRETION OF WATER BY THE NEPHRIC TUBULE OF THE CRAYFISH [1]

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Introduction

When a crayfish is in freshwater, its normal habitat, it does not drink, but water diffuses into its body through the gills (Maluf, 1937, 1940). An internal aqueous and saline steady state is maintained, in spite of a constant inward diffusion of water, because of the unvarying capacity of its kidneys to manufacture urine that is markedly hypotonic to the blood (Schlieper and Herrmann, 1930; Herrmann, 1931).

The concentration of chloride in the luminal fluid of the coelomosac and labyrinth of the nephron is equal to that in the blood, but that in the tubular fluid is markedly lower than that in the blood (Peters, 1935). The hypotonicity of the urine must therefore be due either to an active resorption of salts by the tubule or to an outward secretion of a hypotonic liquid by the tubule.

The tubule of a 35-gram animal is approximately 3 cm. long and about 2 mm. in greatest breadth.[2] The ventral, i.e., proximal, half of the tubule consists of flat cells without apical secretory globules. The dorsal, i.e. distal, coil is composed of relatively large columnar cells with a distinct mitochondrium and, generally, with large clear apical vacuoles which bulge into the lumen of the tubule (Maluf, 1939).

Because the nephron of the crayfish does not possess a tenuous syncytium, such as the glomerular capsule of the vertebrate nephron, filtration seems unlikely as a major process of urine-formation. Accordingly, this is an attempt to find whether the apical vacuoles of the distal coil of the tubule represent an outward secretion of water.

The experimental attack is partly based on the observation of Herrmann (1931) that, as the salinity of the external medium is raised, the rate of urinary flow falls and the osmotic pressure of the urine simul-

[1] This work was performed when the author was Johnston Research Scholar in the Department of Zoölogy, The Johns Hopkins University. To Professor S. O. Mast much obligation is due for numerous kindnesses.

[2] In the 1939 paper this was misprinted as "2 cm."

taneously increases. As shown by constancy in weight, the total quantity of water in the crayfish is the same in freshwater as in salinities up to 272 mM. NaCl per liter, which is initially hypertonic to the blood. This indicates that the decrease in the rate of urinary flow, with rising external salinity, is not due to a decrease in haemocoelic pressure which, assuming that filtration does occur, might cause a decrease in the rate of filtration. There is, furthermore, no apparent basis for the supposition that the haemocoelic pressure undergoes a localized fall in the vicinity of the kidneys as the salinity of the external medium is raised.

From the above it might be expected that, when the crayfish is in a medium in which inward diffusion of water can be only very small and in which the rate of urinary flow is accordingly depressed, the apical vacuoles of the nephric tubule will tend to disappear.

Methods

The test animals were immersed in 210 mM. NaCl per liter of freshwater, a solution in which they can remain vigorous indefinitely. Although this concentration is somewhat hypertonic to the blood at the outset (see Lienemann, 1938, for the normal osmotic pressure of the blood of *Cambarus clarkii*), some water, probably only a negligible quantity, diffuses inwardly because, as Herrmann (1931) showed, the osmotic pressure of the blood eventually exceeds that of the external medium. Parenthetically, the invariable hypertonicity of the blood, as compared with the external medium, is probably mainly because the urine is always hypotonic to the blood regardless of the osmotic pressure of the external medium (Herrmann). Integumental uptake of salt from the exterior is a relatively minor factor, as can be readily calculated (data of Lienemann, 1938, and Maluf, 1940).

At the end of one to several days the animals were sacrificed and their kidneys removed with minimum handling and fixed in unneutralized formol-sublimate for several hours, washed in running tap-water overnight, dehydrated with dioxane (50 per cent, 75 per cent, and two changes of 100 per cent), imbedded in paraffin with a melting point of about 49° C., sectioned 8 μ thick, and stained with eosin and methylene blue-borax.

Results

A seven-day stay of three animals in 210 mM. NaCl per liter abolished almost all the apical vacuoles from the cells of the distal coil of the nephric tubule (Fig. 1) whereas the majority of the corresponding cells of the three controls, which had been in freshwater, possessed large

apical vacuoles which bulged into the lumen of the tubule (Fig. 2). One of the test animals exhibited an exceptional number of vacuoles for an animal in 210 mM. NaCl but the vacuoles were small and scanty as compared with those of the controls. The photographs were taken from areas at random. The data were analyzed objectively as follows: In this experiment two to three slides were prepared containing serial sections of the pair of kidneys from each individual (16 slides in all); the labels were covered so as to remove every vestige of external identification; the slides were shuffled. The examination of each slide never exceeded one or two minutes and was made under low power (100 ×). In 15 slides out of 16, the identification of the series to which the preparation belonged (freshwater or 210 mM. NaCl) was correct. Measurements did not show a correlation between the height of the cells and the existence of apical vacuoles.

The experiment was repeated with six larger animals and a duration of three days. Here, too, the difference between the three test animals in 210 mM. NaCl per liter (Fig. 3) and the controls (Fig. 4) was pronounced. The objective analysis, identical with that above described, showed a correct identification of 20 slides out of 22. Here, too, extensive measurements indicated no correlation between the height of the cells and the presence of vacuoles. The three-day experiment was repeated with confirmatory results: the two test animals showing practically no apical vacuoles whereas the two controls displayed apical vacuoles in the majority of cells of the distal half of the tubule.

Even a 24-hour stay in 210 mM. NaCl produced a practically complete abolition of the apical vacuoles (Fig. 5) although the interior of the cells was considerably vacuolated. Nearly all of the corresponding cells of the controls in freshwater exhibited large clear apical vacuoles (Fig. 6). Figures 5 and 6 are at a lower magnification than the other photographs and thus exhibit a larger field. The objective analysis showed a correct identification of 8 slides out of 8. Subjection to 210 mM. NaCl for less than 24 hours was not attempted.

After vacuole-formation has presumably been practically abolished by an 168-hour stay in 210 mM. NaCl, the vacuoles reappear upon returning the crayfish to freshwater. In this experiment there were two tests and two controls.

The fact that a large fraction of the cells of the distal half of the tubule of the controls invariably exhibited large apical vacuoles in itself shows that the almost complete absence of such vacuoles in slightly hypertonic NaCl is not an artefact of histological technique. The distal half of the tubule of a live animal was dissected out of the kidney in

crayfish-saline.[3] Fragments teased out of this part of the tubule and suspended in a hanging drop of crayfish-saline on a coverslip, gave an ample picture of the vacuoles.

Historical Statement and Discussion

The nephric tubule of the decapod kidney was first discovered by Neuwyler (1841), who did not understand the function of the "green glands." Only within the present decade have we come to realize the importance of the crustacean kidney in the aqueous and ionic regulation of the bodily fluids. Grobben (1881) was the first to observe that the nephric tubules of freshwater Crustacea and Annelida are markedly longer than those of corresponding marine forms and that length of tubule is not correlated with bodily size. He did not theorize as to the significance of these facts but remarked that, "It therefore appears that the length of the urinary canal goes parallel with life in freshwater." Richard (1891) came to an identical conclusion with regard to copepod Crustacea. Rogenhofer (1905, 1909) confirmed Grobben and found that differences in the nephric dimensions of marine and freshwater Crustacea are not due to differences in cellular size. Rogenhofer failed to alter the length of the tubule of the freshwater isopod, *Asellus aquaticus,* in one generation by gradually bringing the isopod to a salinity of 2 per cent in one year. Della Valle (1893) believed that the differences

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PLATE I[4]

Explanation of Figures

Fig. 1. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for seven days. *ha,* haemocoele and blood-vessels; *LU,* lumen of tubule. Triple Mallory's; daylight bulb; Zeiss lens. Animal about 10 grams.

Fig. 2. Control to Fig. 1; animal in freshwater. *va,* large apical vacuoles. Animal about 10 grams.

Fig. 3. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for three days. Methylene blue-eosin; red filter; Zeiss lens. Animal about 30 grams.

Fig. 4. Control to Fig. 2; animal in freshwater. Animal about 30 grams.

Fig. 5. Epithelium of a portion of the distal half of the nephric tubule of a crayfish which had been in 210 mM. NaCl for 24 hours. Methylene blue-eosin; red filter; Zeiss lens. Animal about 13 grams.

Fig. 6. Control to Fig. 5; animal in freshwater. Animal about 13 grams.

[3] The saline was based on the most acceptable data on the concentration of inorganic electrolytes in the blood of the crayfish (see Maluf, 1940, for references) and was as follows (g./1.): NaCl, 7.81; $CaCl_2$, 1.31; $MgCl_2$, 0.82; KCl, 0.70; buffered at pH 7.5 with 0.5 cc. M/5 Na_2HPO_4/NaH_2PO_4. A $\triangle$ of about 0.66° C. is assumed (see Lienemann, 1938, and Schlatter, 1941).

[4] The writer is much indebted to Dr. Charles E. Brambel, The Johns Hopkins University, for kind personal instruction in photomicrography.

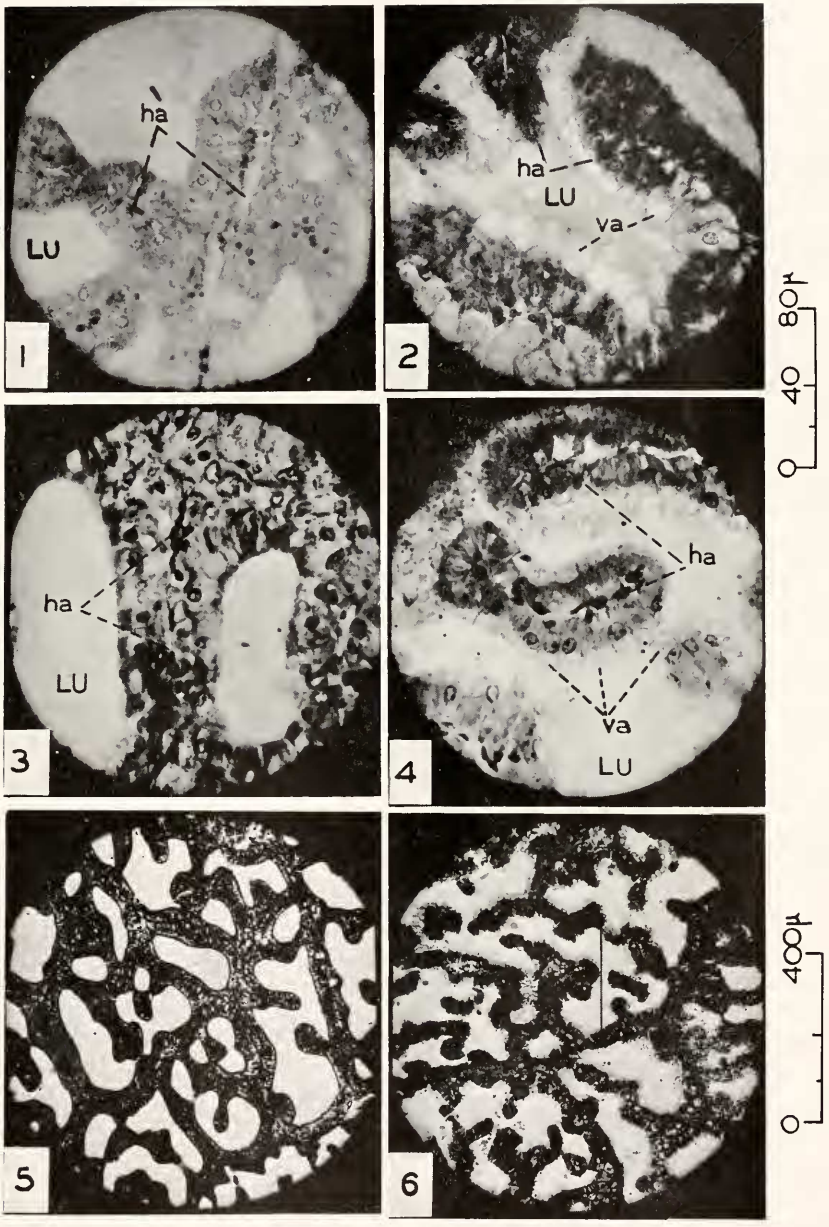

PLATE I[4]

in tubular length are of phylogenetical origin rather than a direct environmental effect. Marchal (1892) observed that the nephrons of the lobster and other marine decapods have no tubule. He suggested that the external medium,—freshwater and sea-water,—may be a determining factor but remarked that the estuarine crab, *Telphusa,* has no nephric tubule even though it frequents freshwater.

In 1930, Schlieper and Herrmann found that the urine of the crayfish is markedly hypotonic to the blood and that the urine of the shore-crab, *Carcinus maenas,* and of the estuarine crab, *Telphusa fluviatilis*—neither of which possess nephric tubules—is isotonic with the blood. They suggested that the nephric tubule is responsible for the hypotonic urine of the crayfish and that it acts by resorbing salts from a filtrate formed at the coelomosac. Herrmann (1931) and Peters (1935), in Schlieper's laboratory, demonstrated that the tubule is of paramount importance in osmoregulation. Peters suggested that the apical vacuoles of the distal coil may indicate a resorption of salts from lumen to blood. Peters' theory presupposes that a filtrate is formed somewhere in the nephron proximal to the tubule. Peters made the important discovery that only in the tubule is the concentration of chloride of the presumptive urine lower than that of the blood. His results do not show, however, in which part of the tubule this is true.

The facts in this paper suggest that the vacuoles represent an outward secretion of water in compensation for that which diffuses inwardly. Physiological data indicate that the crayfish nephron is paramountly if not entirely a secretory organ (Maluf, 1941). The hypotonic urine of this animal may thus be the result of an outward secretion of a liquid markedly hypotonic to the blood and the rate of water-secretion by the tubule may be determined by a hormone.

Summary

The majority of the cells of the distal half of the nephric tubule of the crayfish exhibit large, clear apical vacuoles at their luminal borders when the animal is in freshwater, its normal medium.

If the crayfish remains in a saline medium which is initially slightly hypertonic to the blood, for twenty-four hours or more, these vacuoles completely disappear. The condition is reversible upon return of the animal to freshwater. (The crayfish can maintain its vigor indefinitely in 210 mM. Nacl per liter, which is initially slightly hypertonic to the blood.)

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